

Characterisation of *Pasteurella multocida* associated with bovine respiratory disease in Hungary

Barbara Ujvari¹, Boglarka Seilyei¹, Tibor Magyar¹

1. Institute for Veterinary Medical Research, CAR, HAS, Budapest, Hungary

Pasteurella multocida is a Gram-negative bacterium with a broad host range. This opportunistic pathogen is a common inhabitant of the upper respiratory tract of various mammal and avian species. In the presence of predisposing factors, it causes several diseases, which lead to major welfare problems and remarkable economic losses. The association of *P. multocida* and bovine respiratory disease complex (BRDC) has been well known since the early 1950s. Despite the using of efficient vaccines against other respiratory pathogens, the increased number of cases suggests that *P. multocida* have a definite role in the etiology of BRDC. The aim of our study was to characterize 39 *P. multocida* strains of bovine origin isolated from different herds in Hungary. The studied strains represented only three serotypes: A:3, A:3,4, and A:4. The most frequent biovars, determined by their sugar fermentation abilities and enzyme activities, were biovar 2 (33%) and 3 (44%). Their subspecies were defined with biochemical tests and molecular methods (M13 PCR, 16S rDNA PCR-RFLP). These methods delineated two dominant groups (I and II). Group I consisted of strains belonging to biovar 2, they showed α -glucosidase activity and presented type „B” M13 profile. Whereas group II contained strains with biovar 3, and type „A” M13 profile without α -glucosidase activity. The existence and separation of these groups were confirmed by ERIC-PCR. Looking at the diagnostic background, all strains in group I were associated with pneumonia while the strains in group II originated from various clinical cases. The filamentous hemagglutinin (pftA) that plays an important role in the early colonization of the respiratory tract could only be detected in group I. It may explain why these strains were isolated from cases of bovine pneumonia.

Characterization of lactate utilization and its implication on the physiology of *Haemophilus influenzae*

Sabine Lichtenegger¹, Isabelle Bina¹, Sandro Roier¹, Stilla Bauernfeind², Kristina Keidel², Stefan Schild¹, Mark Anthony³, Joachim Reidl¹

1. Institute of Molecular Biosciences, University of Graz, Graz, Austria

2. Institute of Hygiene and Microbiology, University of Würzburg, Würzburg, Germany

3. Department of Paediatrics, Oxford University Hospitals, Oxford, UK

Earlier work demonstrated that L-lactate metabolism is associated with serum resistance and survival of *Haemophilus influenzae* type b *in vivo*. To gain insight into lactate utilization of non-typeable strains deletion mutants of the L-lactate dehydrogenase (*ldhD*) and permease (*ldhP*) of NTHi 2019 and laboratory strain Rd KW20 were generated and characterized. Comparison of COPD isolate 2019 with the corresponding *ldhP* mutant did not reveal a lactate dependent alteration in serum resistance. Interestingly, we observed a 4-fold attenuation of the mutant strain in a murine model of nasopharyngeal colonization, highlighting a potential role of L-Lactate to persist in this niche. Characterization of *ldhP* transcriptional control shows a negative feedback regulation in the presence of L-lactate, depending on the ArcAB two component system. Additionally, for 2019 it was found that available but not metabolized lactate may have an ArcAB independent signalling function leading to increased cell growth in late log phase. We conclude that L-lactate is not only an important carbon-source but may also act as signal substrate, fine tuning the globally acting ArcAB regulon and potentially a yet unidentified signalling system. Additionally, our data suggest that the ability to take up lactate provides a selective advantage during colonization of the nasopharynx.

Actinobacillus pleuropneumoniae can acquire pyridines compounds from other swine pathogens and form or incorporate into biofilms with other swine pathogens

Abraham Loera Muro¹, Mario Jacques², Francisco Javier Avelar González¹, Josée Labrie², Yannick D.N. Tremblay², Flor Y. Ramírez Castillo¹, Ricardo Oropeza Navarro³, Alma L Guerrero Barrera¹

1. Universidad Autónoma de Aguascalientes, Aguascalientes, AG, Mexico

2. Université de Montréal, Saint-Hyacinthe, QC, Canada

3. Instituto de Biotecnología, UNAM, Cuernavaca, MR, Mexico

Actinobacillus pleuropneumoniae is the etiological agent of porcine contagious pleuropneumonia, which causes important worldwide economic losses in the swine industry. Several respiratory infections are associated with biofilm formation, and *A. pleuropneumoniae* has the ability to form biofilms *in vitro*. Multispecies biofilms are probably the dominant form of microbial growth in nature and clinical data suggest that bacterial species form multi-species biofilms *in vivo*. The goal of this study was to determine the capacity of *A. pleuropneumoniae* to form multi-species biofilms with other swine pathogens (*Streptococcus suis*, *Bordetella bronchiseptica*, *Pasteurella multocida*, *Staphylococcus aureus* and *Escherichia coli*). A serotype 1 field isolate of *A. pleuropneumoniae*, strain 719, was used in a 96-well microtiter plate biofilm assay. *A. pleuropneumoniae* was able to form strong two-species biofilms in the presence of *S. suis*, *B. bronchiseptica* and *S. aureus* under growth conditions that are not favorable for *A. pleuropneumoniae* (BHI media without NAD supplementation) when both species were added at the same time. When inoculated with *P. multocida* or *E. coli*, *A. pleuropneumoniae* biofilm formed a weak biofilm. In all cases, viable bacteria were recovered from the two-species biofilms. The live and dead populations, and the matrix composition of the multi-species biofilms were characterized using fluorescent markers (SYTO-9, propidium iodide, wheat germ agglutinin, Sypro Ruby and BOBO-3) and enzymatic treatments (dispersin B, proteinase K and DNase). The results indicated that poly-N-acetyl-glucosamine remains the primary component responsible for the biofilm structure. *A. pleuropneumoniae* was also able to incorporate into pre-formed biofilms of *S. aureus*, *S. suis* or *E. coli*, under conditions that favour biofilm formation by *S. aureus*, *S. suis* or *E. coli* but not *A. pleuropneumoniae*. In conclusion, our data suggest that *A. pleuropneumoniae* is able to acquire pyridines compounds from other swine pathogens and form or incorporate into biofilms with other swine pathogens.